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Detection of Galactomannan in Bronchoalveolar Lavage Fluid Samples of Patients at Risk for Invasive Pulmonary Aspergillosis: Analytical and Clinical Validity

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Invasive pulmonary aspergillosis (IPA) is frequent and often fatal in immunosuppressed patients. Timely diagnosis of IPA improves survival but is difficult to make. We examined the analytical and clinical validity of galactomannan (GM) testing of bronchoalveolar lavage (BAL) fluid in diagnosing IPA in a mixed population by retrospectively reviewing records of 251 consecutive at-risk patients for whom BAL fluid GM testing was ordered. The performance of the enzyme immunoassay was evaluated by using a range of index cutoffs to define positivity. Three samples were associated with proven IPA, 56 were associated with probable IPA, 63 were associated with possible invasive fungal disease (IFD), and 129 were associated with no IFD. Using a BAL fluid GM index of ≥0.8 (optimal optical density [OD] index cutoff identified by a receiver operating characteristic curve), the sensitivity in diagnosing proven and probable IPA was 86.4%, and the specificity was 90.7%. At this cutoff, positive and negative predictive values were 81% and 93.6%, respectively. However, an OD index value of ≥3.0 corresponded to a 100% specificity, thus ruling the disease in, irrespective of the pretest probability. Conversely, an OD index cutoff of <0.5 corresponded to a high sensitivity, virtually always ruling the disease out. For all values in between, the posttest probability of IPA depends largely on the prevalence of disease in the at-risk population and the likelihood ratio of the OD index value. Detection of GM in BAL fluid samples of patients at risk of IPA has an excellent diagnostic accuracy provided results are interpreted in parallel with clinico-radiological findings and pretest probabilities.

Angio-invasive pulmonary aspergillosis (IPA) is the most common mold infection in immunocompromised patients and causes significant morbidity and mortality. Solid organ transplant recipients and patients with hematologic malignancies, including allogeneic hematopoietic stem cell transplant recipients, are the best-known risk populations (31). However, recently, other frail patient groups have also been identified as risk groups, including critically ill, intensive care unit (ICU) patients. At particular risk are those with advanced liver cirrhosis, those who suffer from chronic obstructive pulmonary disease (COPD) for which they receive corticosteroids, and patients receiving immunomodulating drugs for the treatment of autoimmune disorders (16).

The crude mortality rate of IPA is considerable, though largely influenced by the status of the underlying disease (29, 32). In order to improve the dismal outcome, adequate antifungal therapy needs to be started as soon as possible (33). Hence, it is important to recognize this condition in a timely fashion. However, securing a firm diagnosis of fungal disease is difficult as patients may not exhibit reliable symptoms and signs in the presence of neutropenia and immunomodulating drugs, such as corticosteroids (31). Many centers have now adopted an antifungal treatment approach based on the presence of suggestive lesions on computed tomography scans (such as nodules, with or without a surrounding halo) although this strategy is hampered by a lack of specificity of these radiological features for IPA, especially in nonneutropenic patients (1, 9, 25).

Over the last 2 decades the diagnostic capability for detection of IPA has improved through the use of *Aspergillus*-specific antigens (5). Galactomannan (GM), a major component of the *Aspergillus* cell wall, is released during invasive disease, and the level of circulating serum or plasma GM is indicative of the fungal burden in the host. GM can be detected by the commercially available Platelia *Aspergillus* enzyme immunoassay (GM EIA; Bio-Rad Laboratories, Marnes-la-coquette, France), and results are reported as the optical density (OD) galactomannan index (GMI) (10). Serum or plasma GMI measurements have been studied extensively, gaining widespread acceptance as a sensitive method for prospective surveillance in hematology patients but not in other at-risk groups (17).

More recently, detection of GM in bronchoalveolar lavage (BAL) fluid has been advocated as a sensitive test for diagnosing IPA, both in hematology and nonhematology patients, including solid-organ transplant recipients and critically ill patients (2–4, 7, 8, 13–15, 18, 19, 21–24, 26–28, 30). However, most of the published studies have been restricted to a specific risk group. In addition, the reported literature includes only small numbers of proven and probable cases of IPA. The primary aim of this study was to evaluate the performance characteristics and clinical usefulness of GM detection in BAL fluid for diagnosing IPA in a wide range of underlying at-risk conditions. In addition, we examined

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the performance of this test using different cutoff values for test positivity.

MATERIALS AND METHODS

We conducted a retrospective cohort study at the University Hospitals Leuven (Leuven, Belgium), a tertiary-care university center. All adult patients (≥18 years of age) who underwent a bronchoscopy with lavage between 1 January 2009 and 31 December 2009 and who had BAL fluid tested for GM were reviewed. The study protocol was approved by the Institutional Review Board. All patients gave informed consent before the procedure.

Bronchoscopy was performed using a flexible fiberoptic bronchoscope following local anesthesia with 2% lidocaine (Xylocaine). All procedures were performed with sterile isotonic saline (20 ml twice); no Plasmalyte solution was used (11). The sampling area was determined on the basis of the lesion location on chest imaging (X-ray or computed tomography [CT] scan). Following aspiration, aliquots were immediately sent to the microbiology and pathology laboratories. BAL fluid samples were submitted for cytology assessment, Gram staining, auramine staining, and calcofluor white fungal staining and for aerobic bacterial, fungal, viral, and mycobacterial cultures. In addition, PCR testing for respiratory viruses, *Pneumocystis jirovecii*, *Mycoplasma*, and *Legionella* was performed. During this study period, integrated-care pathways for fungal diagnosis and treatment were operational for hematology patients, solid-organ transplant recipients, and patients admitted to the medical and surgical ICU. These care pathways clearly identified triggers for requesting additional radiological evaluation (including CT scan) and, if indicated, for requesting a bronchoscopy with lavage. As per protocol, detection of galactomannan in lavage fluid was a standard test. For the remaining patients, the test was usually requested by the chest physician who performed the bronchoscopy.

The Platelia *Aspergillus* GM EIA (Bio-Rad Laboratories, Marnes-la-Coquette, France) was used to detect the presence of GM on uncentrifuged BAL fluid specimens. The test was performed according to the manufacturer's recommendations for testing serum samples. The assay was run three times weekly in the routine microbiology laboratory. Pretesting samples were stored at 2 to 8°C. All tests were performed by technicians who were unaware of the clinical condition of the patient.

The following baseline findings were retrieved from the hospital chart: age, gender, underlying disease, department of hospitalization, antifungal use (prophylaxis and treatment), use of corticosteroids, administration of piperacillin-tazobactam or amoxicillin-clavulanate, and neutrophil count at the day of testing (± 1 day). Results of bacterial, fungal, and mycobacterial cultures and of PCR tests performed on BAL fluid samples were retrieved from the laboratory information system; results of direct microscopic examination of BAL fluid and respiratory tissue biopsy samples were collected from the pathology department. All chest CT scans (or chest X-rays when CT scan was not available [mainly for ICU patients]) were reviewed.

The BAL fluid GM EIA results were benchmarked against the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) consensus definitions for invasive aspergillosis (IA) (6). In the absence of histopathology findings, these definitions are considered to be the most appropriate reference standard for assessing the diagnostic performance of new diagnostic tests in medical mycology. The EORTC/MSG categorization classifies the target population as having proven IPA, probable IPA, possible invasive fungal disease (IFD), and no IA. All assessments were done by two independent investigators who were also blinded to the BAL fluid sample GM EIA results.

Proven IPA was defined by positive histopathology from lung tissue. These samples could have been obtained premortem or postmortem. For all patients fulfilling the revised EORTC/MSG host criteria (neutropenia, allogeneic stem cell transplant, prolonged use of corticosteroids, use of immunosuppressive drugs, or inherited severe immunodeficiency), probable IPA was defined by the presence of features of a lower respiratory tract fungal disease on CT scan (only dense, well-circumscribed lesions with or without a halo sign, an air crescent sign, or a cavitated lesion were accepted as radiological abnormalities) and at least one mycological criterion (e.g., positive culture or microscopy for mold from a respiratory sample or at least two positive [OD of ≥0.5] serum GM detections). To avoid incorporation bias, GM detection in BAL fluid was not included as a mycological criterion. As previously stated, critically ill patients with chronic obstructive pulmonary disease, autoimmune disorders, or liver cirrhosis were added as host factors (21). However, because a CT scan is less feasible and because the radiological consensus criteria are less specific for IPA in these groups of patients, the presence of pulmonary infiltrates or consolidations not responding to broad-spectrum antibacterial coverage was considered a sufficient radiological criterion (21). Cases with a host factor and compatible radiological features but without a mycological criterion were considered possible IFD. All other patients were considered IPA negative.

In an attempt to minimize spectrum bias (i.e., excluding important patient subgroups), we have included BAL fluid specimens from all patients for whom the test is intended to be used (the target population); this includes all patients fulfilling the host criteria as defined per revised EORTC/MSG consensus definitions and presenting with unexplained nodular lesions or pulmonary consolidations/infiltrates on chest X-ray or (preferably) chest CT scan. In addition, specific subgroups of ICU patients, namely, those with advanced liver cirrhosis, chronic obstructive pulmonary disease on corticosteroids, and autoimmune disorders receiving immunomodulating therapy with the presence of pulmonary infiltrates or consolidations not responding to broad-spectrum antibacterial coverage were also included. Hence, BAL fluid samples of patients not fulfilling the above-mentioned host criteria, patients without radiological abnormalities (e.g., BAL fluid examined as part of a protocol-defined follow-up procedure), and patients without recent radiological examinations were excluded from the analysis. To avoid disease progression bias for patients with multiple BAL fluid samples available, only the first sample was included in the analysis. Finally, only BAL fluid samples taken before the start of mold-active antifungal therapy were included in the analysis.

For performance analysis, proven and probable IPA cases were grouped together as IPA. Diagnostic performance was expressed as sensitivity, specificity, positive and negative predictive value (PPV and NPV, respectively), positive and negative likelihood ratio (+LH and −LH, respectively), diagnostic odds ratio, and error odds ratio relative to different optical density (OD) index cutoff values by using two-way contingency tables. A 95% confidence interval (CI) was calculated for each value (using the calculator at http://statpages.org/ctab2x2.html). The area under the receiver operating characteristics (ROC) curve was constructed to assess how changes in the OD index cutoff for the GM EIA assay altered sensitivity and the value of 1 minus specificity. Fagan's nomograms were constructed for calculating posttest probabilities (using the calculator at http://araw.mede.uic.edu/cgi-bin/testcalc.pl).

RESULTS

Patient population. During the 1-year study period, a total of 957 first BAL fluid samples were tested for the presence of GM by the Platelia EIA. However, 706 (73.8%) BAL fluid samples came from patients not fulfilling the target population criteria and were excluded from our analysis. The main reason for exclusion was the absence of radiological abnormalities (e.g., BAL fluid samples obtained as part of a follow-up strategy after lung transplant or during investigation of chronic cough). A total of 251 BAL fluid samples (26.2%) were collected from patients fulfilling the target population criteria. Study population characteristics are summarized in Table 1. The majority of patients were solid-organ transplant recipients (29%) and patients with hematologic malignancies (20.3%).

Overall, we identified 59 cases of proven ($n = 3$) or probable

TABLE 1 Patient characteristics

Patient characteristic	Value for the parameter (no. of patients [%])
Total population	251 (100%)
Age distribution (yr)	
18–30	15 (5.9%)
31–40	20 (8%)
41–50	37 (14.7%)
51–60	58 (23.1%)
61–70	68 (27%)
71–80	41 (16.3%)
>80	12 (4.8%)
Male	146
Female	105
Distribution by medical department	
Medical Oncology	12 (4.8%)
Cardiology	4 (1.6%)
Gastroenterology	10 (4%)
Geriatrics	3 (1.2%)
Hematology	38 (15.1%)
General Internal Medicine	12 (4.8%)
Intensive Care	54 (21.5%)
Surgical	16
Medical	38
Nephrology	21 (8.4%)
Neurology	2 (0.8%)
Chest clinic	91 (36.2%)
Rheumatology	4 (1.6%)
Distribution by main underlying condition	
Hematologic malignancy	51 (20.3%)
Stem cell transplant	15
Other treatment	36
Solid organ transplant	73 (29%)
Nonhematologic malignancies	33 (13.1%)
Intensive care admission	35 (13.9%)
Other	59 (23.1%)
Invasive aspergillosis status	
Proven/probable	3/56 (23.6%)
Possible	63 (25.0%)
No invasive aspergillosis	129 (51.4%)
Antifungal prophylaxis status	
No prophylaxis	190 (75.7%)
Fluconazole	28 (11.1%)
Mold-active prophylaxis	33 (13.2%)

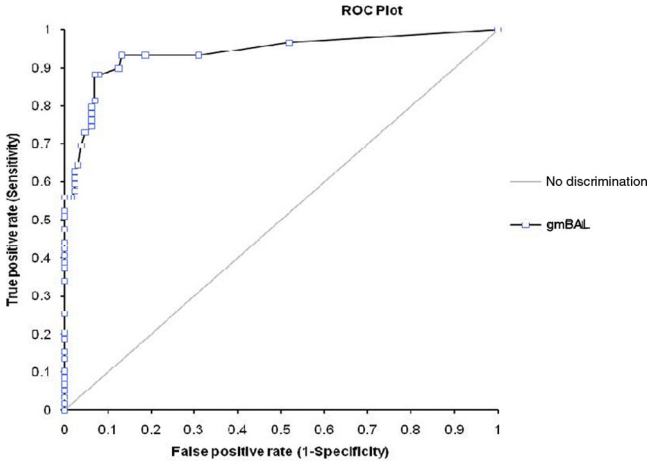

FIG 1 ROC curve for the Platelia EIA in BAL fluid samples.

(n = 56) IPA (23.6%; the case patients). Of these 56 probable cases, 42 had a positive BAL fluid culture for *Aspergillus fumigatus* complex, and one patient presented with a mixed *Aspergillus flavus* and *Aspergillus niger* infection; the remainder had at least two consecutive positive serum GM assays. A total of 129 patients (51.4%) had no evidence of IPA (the control patients). Sixty-three patients (25%) presented with possible invasive fungal disease; these cases were excluded from the performance analysis because the true nature of the pulmonary lesions could not be adequately determined.

GM assay results. The performance of GM detection in BAL fluid samples was calculated for different OD cutoff indices; these results (with 95% confidence intervals) are summarized in Table 2. The sensitivity-specificity analysis showed an inverse relationship: the higher the proposed cutoff index, the higher the specificity and the lower the sensitivity (and vice versa). An OD index value of ≥3.0 corresponded to a 100% specificity; thus, a positive result ruled the disease in since there were no false-positive results. Conversely, an OD index value of <0.5 corresponded to a very high sensitivity; as such, a negative result virtually always ruled the disease out since there were very few false negatives. In contrast, culturing of BAL fluid samples had a sensitivity of 72%.

To determine the most appropriate OD index cutoff to define positivity, a ROC curve was calculated (Fig. 1), indicating 0.8 as the most optimal cutoff value for this mixed patient population.

TABLE 2 Performance characteristics of the Platelia galactomannan enzyme immunoassay in bronchoalveolar lavage fluid

OD index cutoff	Value for the parameter (95% CI)[a]							
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	−LR	DOR	EOR
≥0.5	93.2 (85.7–97.2)	86.8 (83.4–88.6)	76.4 (70.2–79.6)	96.6 (92.7–98.6)	7.0 (5.1–8.5)	0.07 (0.03–0.17)	90.5 (29.9–270.3)	2.0 (1.1–4.4)
≥0.80	86.4 (77.4–92.7)	90.7 (86.6–93.6)	81 (72.5–86.8)	93.6 (89.3–96.5)	9.29 (5.75–14.3)	0.15 (0.07–0.26)	62.15 (22.0–183.9)	0.65 (0.53–0.87)
≥1.0	79.7 (71.6–85.2)	93.8 (90.1–96.3)	85.5 (76.8–91.4)	91 (87.4–93.5)	12.8 (7.2–23.3)	0.21 (0.15–0.31)	59.2 (23–152.3)	0.26 (0.27–0.21)
≥1.5	72.9 (65–77.9)	95.3 (91.7–97.6)	87.8 (78.2–93.8)	88.5 (85.1–90.6)	15.6 (7.8–33.1)	0.28 (0.22–0.38)	55 (20.5–146.5)	0.13 (0.16–0.08)
≥2.0	62.7 (55.6–66)	97.7 (94.4–99.2)	92.5 (81.9–97.3)	85.1 (82.3–86.4)	26.9 (9.9–80)	0.38 (0.34–0.41)	70.6 (21–233.5)	0.04 (0.07–0.01)
≥2.5	59.3 (52.1–62.6)	97.7 (94.4–99.2)	92.1 (81–97.2)	84 (81.2–85.3)	25.5 (9.3–76)	0.41 (0.37–0.5)	61.2 (18.3–201.8)	0.03 (0.06–0.01)
≥3.0	55.9 (50–55.9)	100 (97.6–100)	100 (90.6–100)	83.2 (81.2–83.2)	∞ (21–∞)	0.44 (0.44–0.5)	∞ (41.5–∞)	0 (0.02–0)
≥4.0	47.5 (42.2–47.5)	100 (97.6–100)	100 (88.9–100)	80.6 (78.7–80.6)	∞ (17.5–∞)	0.52 (0.52–0.59)	∞ (29.6–∞)	0 (0.02–0)

[a] Values are for proven and probable cases versus controls (n = 188). CI, confidence interval; OD, optical density; PPV, positive predictive value; NPV, negative predictive value; +LH, positive likelihood ratio [sensitivity/(1 − specificity)]; −LH, negative likelihood ratio [(1 − sensitivity)/specificity]; DOR, diagnostic odds ratio {[sensitivity/(1 − sensitivity)]/[(1 − specificity)/specificity]}; EOR, error odds ratio {[sensitivity/(1 − sensitivity)]/[specificity/(1 − specificity)]}.

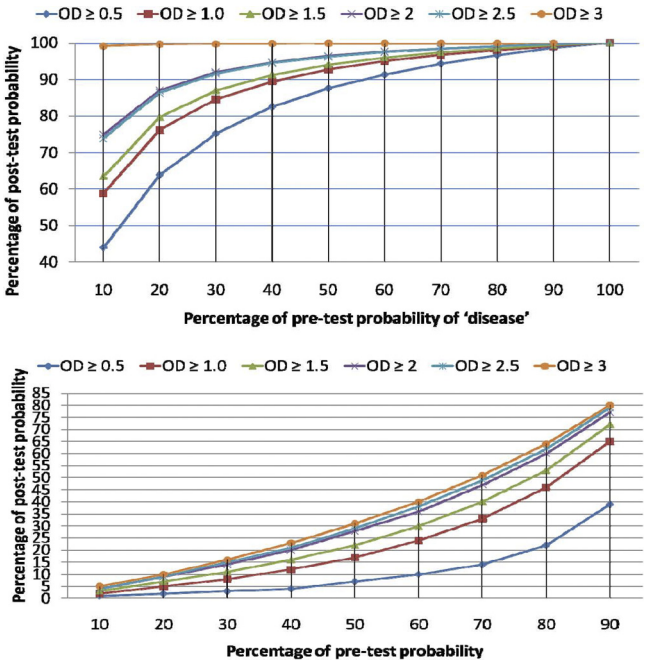

FIG 2 The posttest probability of disease was calculated by multiplying the prevalence (or pretest probability, ranging from 0 to 100%) by the likelihood ratios for the different OD index cutoff values (as depicted in Table 2); these results are plotted as positive likelihood (top) and negative likelihood (bottom).

The diagnostic accuracy as given by the area under the ROC curve was 0.94 (standard error, 0.021; 95% CI, 0.90 to 0.98). The performance of the assay in BAL fluid samples, using this cutoff of 0.8, is shown in Table 2. The performance was not different in neutropenic versus nonneutropenic patients ($P = 0.31$, Fisher's exact test). We also identified 63 patients with possible fungal disease although they were not included in the final analysis. Galactomannan titers of ≥0.8 were detected in the BAL fluid of nine of these patients (14.3%). The optical density index values ranged between 0.8 and 6.7.

However, sensitivity and specificity measure the analytical validity of the test (the accuracy and precision). For an evaluation of the clinical validity (i.e., the accuracy with which a test identifies or predicts a clinical status), we rely on the predictive values and the diagnostic odds ratio. The positive predictive value of the GM BAL fluid assay is excellent (100%) at an OD index cutoff of ≥3 but only moderate (76.4%) when a cutoff of ≥0.5 is used. However, the latter cutoff is associated with a very high negative predictive value (96.6%). Unfortunately, predictive values depend on the prevalence of the disease under study in a given population; as such, predictive values may not apply to the entire target population. To bypass differences in prevalence, we calculated the positive- and negative-likelihood ratios since these values depend less on the prevalence of IPA in the population (Table 2). In addition, we assessed the posttest probability of disease by multiplying the prevalence (or pretest probability, ranging from 0 to 100%) by the likelihood ratios for the different OD index cutoff values; these results are plotted in Fig. 2 as positive likelihood (top) and negative likelihood (bottom). This analysis shows that even in a population with a low prevalence (e.g., 10%), a high value of the GM assay (OD index of ≥3.0) implies a posttest probability of almost 100%. Conversely, in a low-prevalence population, a positive test using a cutoff of 0.5 will not be helpful to guide the clinical decision to start or withhold antifungal treatment. When we modify the diagram for the probability of absence of disease (Fig. 2B), we can conclude that a negative test in a population with a low, moderate, or even high prevalence of IA implies a significant decrease in posttest probability.

DISCUSSION

Pulmonary involvement is a hallmark of invasive aspergillosis. Bronchoscopy with examination of BAL fluid is therefore widely used for the evaluation of patients with suspected invasive aspergillosis, relying on diagnosis by culture and visualization of fungal elements microscopically using specific stains (20). However, these methods are often falsely negative and time-consuming in the case of isolation and identification by culture (12). Hence, a culture-independent method may increase the yield of BAL fluid samples. Recently, the clinical utility of GM EIA detection using BAL fluid samples has been explored in a variety of adult and pediatric populations; these studies, which use various OD cutoff indices for positivity, have reported high sensitivities in hematology patients (2, 3, 7, 13, 19, 22, 24, 26, 28, 30), most solid-organ transplant recipients (4), critically ill patients (21), and nonimmunocompromised patients (23). However, significantly lower sensitivities have been reported in lung transplant recipients (4, 15, 18, 27). A recent meta-analysis determined the sensitivity to be 90% and the specificity to be 94%. Moreover, a positive result increased the probability of having IPA about 6-fold, whereas a negative result decreased the probability to 1% (10).

This large-cohort study assessed the clinical utility of the BAL fluid GM EIA in a mixed population of at-risk patients who underwent bronchoscopy with BAL to evaluate the etiological nature of unexplained pulmonary abnormalities. The study represents real-world observations. The sensitivity of the BAL fluid GM EIA using an OD cutoff value of ≥0.5 was 93.2%; as such, a negative BAL fluid GM assay value virtually excludes the presence of IPA, irrespective of the pretest probability of disease. This high sensitivity is, however, offset by a reduction in test specificity. Using an OD cutoff value of 0.5, at least in patients not receiving mold-active drugs, results in a significant rate of false-positive results. As such, an OD index of ≥0.5 does not confirm the diagnosis of IPA and requires further investigation. However, a negative result makes the diagnosis very unlikely. This is furthermore underscored by the low negative likelihood ratio (below 0.1) at this cutoff, providing convincing evidence to rule out diagnosis in most circumstances. On the other hand, an OD index value of ≥3.0 corresponds with an excellent specificity, securing the diagnosis of IPA, but at the expense of a reduced sensitivity. Again, the high positive likelihood ratio (above 10) at this cutoff provides convincing evidence to rule in diagnosis in most circumstances. We therefore propose to use this OD index cutoff to include patients in clinical studies evaluating the efficacy of antifungal drugs in proven and probable cases of IPA. However, patients with possible IFD may also be candidates for antifungal therapy. Hence, for daily clinical practice, a lower OD index cutoff value (0.8) seems to be much more appropriate, as evidenced by the ROC analysis. This lower cutoff is also in line with previously published experiences (24). Of note, at these lower values, the pretest probability of disease remains crucially important for determining the posttest probability (Fig. 2).

Our study has several limitations. First, physician-driven decisions for performing bronchoscopy and the timing of bronchoscopy are potential sources of bias. This is certainly the case for patients not treated according to integrated care pathways (1). As a consequence, samples may have been collected at various time points during the course of the infection and at various stages of fungal disease. Second, BAL protocols, contrary to serum sampling, are not standardized; the variable amount of instilled and collected fluid may affect GM concentrations and, consequently, the sensitivity of the assay, as evidenced in a recent study (28). Normalizing BAL fluid GM values to another lung fluid analyte (such as urea) could be a way of circumventing this problem in future studies. Third, by using the EORTC/MSG consensus definitions, we used a very conservative (but generally accepted) reference standard to estimate the performance characteristics of this assay. Unfortunately, the 2008 revised definitions fail to capture all cases of IPA, both in patients with the appropriate host factors (e.g., patients with airway-invasive aspergillosis presenting with radiological features not fulfilling the EORTC/MSG criteria) and in patients without appropriate host factors and clinical features (in particular, ICU patients and many solid-organ transplant recipients) (9, 21, 25); therefore, the specificity of BAL fluid GM detection at various cutoff values may be underestimated. Fourth, this study cannot assess the impact of antifungal prophylaxis or therapy on the test performance since too few patients received mold-active prophylaxis and since BAL fluid samples were collected before the start of antifungal therapy. However, according to recent data, treatment with antifungal therapy for two or more days promptly decreases the assay sensitivity in BAL fluid samples as well as in serum samples (28). Fifth, because of the retrospective nature of the study, we cannot provide data on the diagnostic performance of the GM assay in BAL fluid samples in comparison with paired serum assays.

The strength of this study could be the high number of documented cases, the minimization of spectrum bias, and the external validity of the findings. Although the number of patients with proven IPA is small, this study included a substantial number of patients with well-documented probable IPA. Estimates of diagnostic accuracy are usually subject to spectrum bias because most studies do not include the complete spectrum of subgroups at risk, frequently omitting the intermediate and typically more difficult cases to diagnose (e.g., critically ill patients in intensive care units and COPD patients). Eliminating these cases produces an overly optimistic picture of how the assay performs in clinical practice. So, we aimed at estimating the performance of a BAL fluid GM EIA in a population sufficiently representative of the intended-use population, including traditional as well as emerging risk groups for IPA. As such, we could also show that the performance of the assay in BAL fluid samples—in contrast to results with blood samples—was not influenced by the neutropenic status of the host. Finally, we believe that these results are reflective of the real-world performance of the BAL fluid GM EIA. Indeed, over this 1-year period, several batches of the assay have been used by multiple trained and experienced technicians in a nonresearch, clinical microbiology laboratory.

In conclusion, detection of GM in BAL fluid samples of patients at risk of invasive pulmonary aspergillosis (and before receiving mold-active drugs) can be used as a diagnostic method with excellent accuracy, provided results are interpreted in parallel with clinico-radiological findings and pretest probabilities. Future research should primarily focus on the impact of preanalytic variables (e.g., volume of BAL fluid) and standardization of the bronchoscopic procedure.

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